Chapter

Nanotechnology Application and Intellectual Property Right Prospects of Mammalian Cell Culture

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Abstract

The significant challenges faced by modern-day medicine include designing a target-specific drug delivery system with a controlled release mechanism, having the potential to avoid opsonization and reduce bio-toxicity. Nanoparticles are materials with nanoscale dimensions and maybe natural and synthetic in origin. Engineered nano-sized materials are playing an indispensable role in the field of nanomedicine and nanobiotechnology. Besides, engineered nano-sized particles impart therapeutic applications with enhanced specificity because of their unique bespoke properties. Moreover, such application-customized nanoparticles offer an enormous possibility for their compatibility with different biological molecules like proteins, genetic materials, cell membranes, and organelles at the nano-bio frame. Besides, surface functionalization with targeting moieties such as small molecule ligands, monoclonal antibodies, aptamers, cell-penetrating peptides, and proteins facilitate nanoparticle-based specific tissue targeting. This review summarizes some of the advances in nanoparticle-based therapeutics and theranostics. A better understanding of idealistic preparation methods, physicochemical attributes, surface functionalization, biocompatibility can empower the potential translation of nanomaterials from the 'bench-to-bedside'. In modern-day medicine, engineered nanoparticles have a wide range of demands ranging from bio-imaging, theranostics, tissue engineering, sensors, drug and nucleic acid delivery, and other pharmaceuticals applications. 2D and 3D mammalian cell-based assays are widely used to model diseases, screening of drugs, drug discovery, and toxicity analyses. Recent advances in cell culture technology and associated progress in nanotechnology have enabled researchers to study a wide variety of physiologically relevant questions. This chapter explores the properties of nanoparticles, different targeted delivery methods, biological analysis, and theranostics. Moreover, this chapter also emphasizes biosafety and bioethics associated with mammalian cell culture and discusses the significance of intellectual property rights from an industrial and academic perspective.

Keywords: nanotechnology, intellectual property right, mammalian cell culture, nanoparticle biocompatibility, targeted drug delivery, bioethics

1. Introduction

Nanomaterials (NMs) are engineered chemical substances or materials with a particle size of 1–100 nm in diameter. Today NMs are extensively explored and engaged for commercial purposes in different fields, and many sophisticated NMs have shown great promise in biotechnology and biomedicine [1]. NMs display inimitable physicochemical attributes due to their size range in nanometers, high surface area, tunable surface charge, unique composition, various morphologies, and surface composition. Due to their remarkable physicochemical attributes, NMs are significantly different from their bulk materials of a similar symphony, allowing them to perform remarkably well with improved functionality, sensitivity, competence, and selectivity towards developing biomedicines. Various NMs are evaluated to get desired biomedical efficacy for nanomedicine-related applications, including different metal nanoparticles, liposomes, quantum dots, polymeric micelles, dendrimers, and carbon-based nanoparticles. Two critical mechanisms for delivering drug-loaded NMs to the diseased sites are passive targeting and active targeting. A passive targeting mechanism happens via enhanced permeability and retention (EPR) [2]. Inactive targeting mechanism relies on surface functionalized NMs with various biomarkers that bind with receptors over-expressed at the pathological tissue [3].

The importance of cell culture advances in the medical sector has long been recognized. Mammalian cell culture (MCC) entails first isolating cells from a specific organ tissue and then creating a culture in a suitable artificial setting. Disaggregation using different methods may be used to obtain preliminary separation of cells from the identified organ tissues. The isolated primary cells are typically obtained from an *in vivo* setting, although some cells come as established cell lines. MCCs are widely used in the biomedical field to investigate numerous applications [4]. Since cell culture-based studies provide highly stable and repeatable results, researchers consider this technique as an essential model system in cellular and molecular biology. MCC needs an ideal environment for development, which can be divided into nutritional and physicochemical requirements.

Nutritional necessities comprise an adherent substrate or growing medium that offers conditions like essential amino acids, sugars, vitamins, minerals, growth factors, hormones, and gases (O_2, CO_2). All these features regulate physicochemical factors such as pH, osmotic pressure, and temperature. Many cell lines need solid or semi-solid support in the form of a substrate, while others can be grown in a suspension culture medium. These technologies have evolved as a means of assessing the efficacy and side effects of novel active pharmaceutical ingredients (APIs), immunotherapeutic, and biopharmaceuticals [5]. Animal, plant, and bacterial cells are regularly cultured in fixed culture medium under precise laboratory circumstances; among this, animal-based cell cultures are more complex than others due to their genetic complexity. Directed differentiation of adult stem cells and pluripotent stem cell culture is another challenging aspect. Recent advances in stem cell culture technology have provided significant input for the successful culture of tissue-mimicking 3D organoids [4, 6].

In recent years, nanotechnology (NT) and associated disciplines have gained rapid escalation in biomedical implementations such as diagnosis, testing, tracking, drug

delivery, nanomedicine, medical implants, and electronics due to their camaraderie with biological entities. Biomedicine embraces the design and synthesis of NMs, along with other nanoparticles (NPs) and nano-devices [7]. Once properly formulated, NMs show their natural aptitude to traverse with the blood flow via various routes based on their attributes and eventually get access to all the organs. Due to their intrinsic biocompatible interactions, the NPs exhibit unique physicochemical attributes associated with lesser immunogenicity and non-toxicity. There are numerous advantages of using NMs for various biological applications: i) it increases the concentration of drug in the pathological tissues and control the slow release of the drug; ii) it solves issues connected to the low solubility and bioavailability of the drug; and iii) enhanced biodegradability and biocompatibility iv) drugs/genes/imaging agents can be easily loaded due to their tunable surface functionalities [1, 7, 8]. Imaging agents could endow *in vivo* drug tracking ability to determine drug delivery efficacy during treatment. In recent years, various nanoparticles such as liposomes, polymers, metal nanoparticles, inorganic nanoparticles have been developed for selectively targeting tumor cells and other pathological tissues without causing any destruction to healthy cells or organs. In this chapter, the application of nanotechnology and Intellectual property rights (IPR) prospects of mammalian cell culture will be discussed in the subsequent sections.

2. Compatibility of nanomaterials towards biological interactions

NMs attract considerable interest due to their unique, tunable, versatile physicochemical properties, easy preparation methods, biocompatibility, and surface functionalization [1]. Nonetheless, the compatibility of the nanoparticles with biological entities constitutes the most fundamental phenomenon and highlights the importance of basic research [9]. Most bio-applications, including drug delivery, bioimaging, and treatment, start from the attachment of nanoparticles onto the target cells. The biocompatibility of nanoparticles depends on the physical and chemical attributes like diameter, shape, composition, concentration, functionalized moieties, and surface potential (**Figure 1**) [10]. Among the various NMs, Quantum dots have risen as an innovative bio-imaging tool due to their unique tunable physicochemical attributes. Existing research has guided the development of versatile quantum dots that are highly fluorescent and stable under diverse biological circumstances. Moreover, quantum dots with enclosed amphipathic polymers have been developed and surface-functionalized with receptor targeting ligands for bio-imaging and drug-delivery in animal models. Fascinatingly, these materials were found to be compatible with the cells. However, their complete chronic *in vivo* genotoxicity, blood, and organ compatibility need to be assessed [1, 7, 11].

Polymeric nanoparticles have drawn considerable attention in drug and gene delivery, tissue engineering, and many biomedical applications due to their non-toxic nature and high compatibility to biological systems. They are colloidal in nature and composed of natural or synthetic, or semi-synthetic polymers. In this perspective, biodegradable nanoparticles of the highly compatible triblock copolymer are used for non-viral gene transfections [3].

Liposomes are another popular nanomaterial drug delivery system that is best documented and adapted owing to their bio-congenial physicochemical properties [12]. Liposomes consist of unilamellar/ multilamellar lipid bilayers having an aqueous core inside. The nanoscale carrier system offers substantial advantages such as

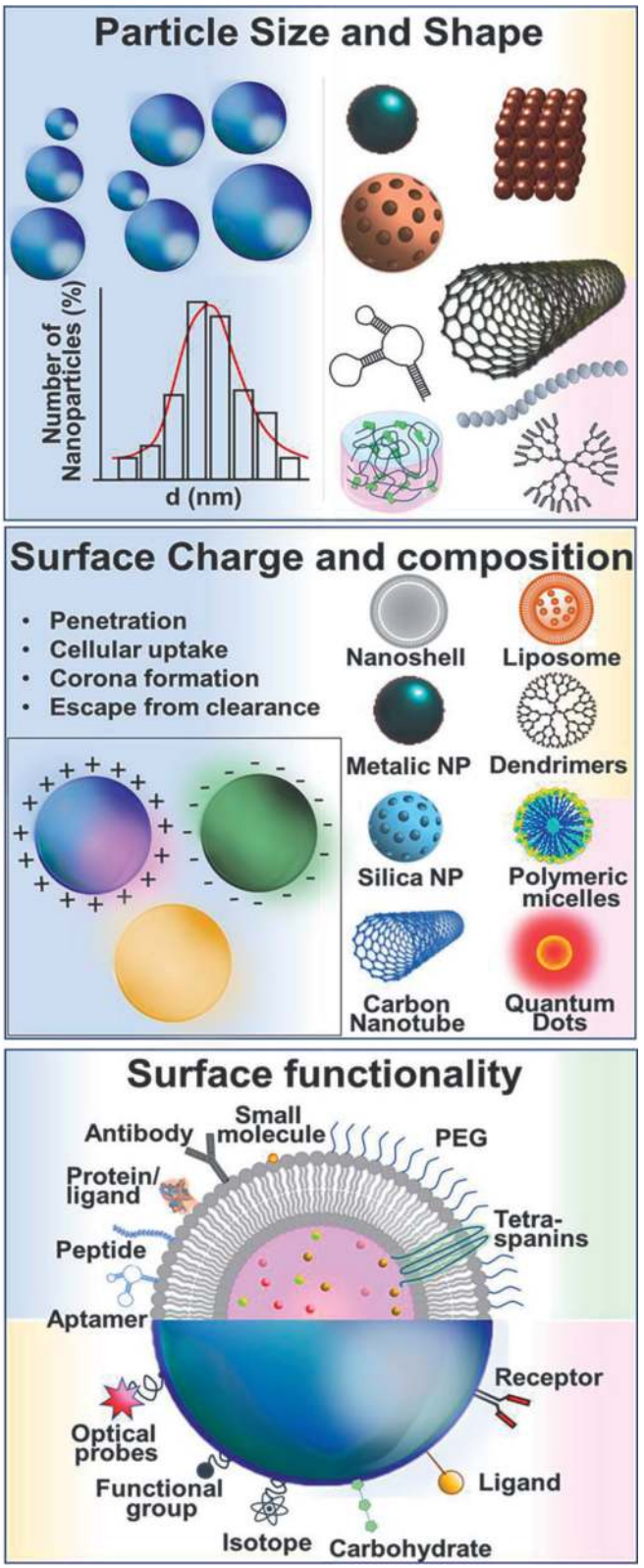

Figure 1.
Precision of targeted drug delivery using nanocarriers and bio-compatibility. Nanoparticle based drug delivery platform depends on surface functionality, size and shape and surface charge and composition.

biodegradability, biocompatibility, ease of synthesis, less toxicity, sustained drug release, and the ability to incorporate hydrophilic and hydrophobic drugs. Liposomal-surface modification is a crucial strategy for targeted therapy and especially for cancer treatment [13]. Seventeen liposomal formulations are clinically approved for cancer, inflammation, infectious diseases, antibiotic drugs, and anesthetics, while several liposomal formulations are under various phases of clinical trials [12, 13].

Despite several encouraging biomedical implementations of nanoparticles, the biocompatible assessments including, complete acute and chronic toxicological evaluation of NMs, are inadequately comprehended. Additionally, the toxicity of the nanoparticle design aims to find out favorable physicochemical attributes of different materials. Hence, the active bio-molecule with biological entities must be highly allied to the nanoparticles approaching direct contact with biological objects rather than its transient initial distribution. Much to our intrigue, various nanoparticles - liposomes, lipoplexes, polymeric nanoparticles, polyplexes, metal nanoparticles, metal oxides, dendrimers, and quantum dots are wisely engineered for their medical application like diagnosis, drug and gene delivery, tissue engineering, and biosensing [8, 13, 14]. Moreover, it is unavoidable to thoroughly assess and investigate compatibility/unwanted toxicity with nanoparticles to bring clinical success. The subsequent section will relate to how the physicochemical properties of engineered nanoparticles can be persuaded towards accomplishing the desired biological aspiration lacking any toxicological impact.

2.1 Tunable physicochemical attributes of nanomaterials compatible with biomedical applications

Nanoparticles exhibit outstanding physicochemical attributes which can be manipulated to harness the best possible benefits out of them - their tunable diameter, high surface area, various morphologies, different concentrations and compositions, surface functionalization, etc. [15] (**Figure 1**). Interactions of NMs to the cell surface, their internalization, and subcellular localization, communication with the cells eventually contribute to therapeutic or adverse effects. Understanding the physicochemical attributes of NMs and their interactions with biological entities can help design superior NMs for further applications. We are jotting down the relevant physicochemical attributes of nanoparticles, which may modulate their function in therapeutic or toxicity aspects; thus, they need to be engineered wisely [16].

2.1.1 Nanomaterials size

For engineered nanoparticles, the primary crucial feature is their dimensions/size, which partially governs other physicochemical characteristics. The reduced diameter of the nanoparticles, provide possibilities for high cellular localization making them interact with cellular tissues, especially pathological tissue to a greater extent to attain specific biological outcome for the remedial purpose. Size-dependent bio-distribution studies were performed using three different sizes containing (20, 50, and 200 nm) drug conjugated silica nanoparticles. It revealed that nanoparticles having 50 nm diameter had the highest tumor localization, enhanced cancer tissue retention, and slower clearance [16]. Moreover, nano-sized particles preside over their pharmacokinetics, are predictable to traverse biological barriers, which is not possible for bulk

particles. Besides, ~50 nm diameter particles showed higher efficacy because of active engagement to the biological tissues, modulating pathways, and cellular activities [17].

2.1.2 Nanomaterials surface charge

The surface charge is a unique character of NMs to manage its therapeutic and toxicological effects and plays a significant role in electrostatic interactions of NMs and living entities (**Figure 1**) [10]. Besides, the cellular localization pathways and tissue interactions are regulated by the surface charge of the nanoparticles, thus playing a significant role in the compatibility and cellular toxicity. Several reports suggest that nanoparticles with a positive charge highly interact with the negatively charged cell membranes and provoke genotoxicity [18]. Positively charged cationic liposomal drug and gene delivery systems have been extensively studied for the last decade. It was recently shown that cationic lipoplexes are not showing any genotoxicological aberrations in the Swiss albino mice. Typically, cell membranes are anionic in nature; thus, negatively charged NMs have very slow cellular internalization compared to neutral and positive nanoparticles [14]. Surface potentials of metal particles in regulating different tumorous and non-tumorous tissue types are also established. Several studies have suggested the role of the surface potential of different nanoparticles and their interactions with the biological entities and how surface charge modulates their biological functions, which shed light to design and engineer nanoparticles for a selective cellular target for various diseases with minimal toxicity [16, 18].

2.1.3 Surface functionalization

Nanoparticles play a vital role in promoting intracellular delivery of encapsulated therapeutic agents and increase their retention in pathological tissues compared to healthy tissues [1]. Surface functionalization with suitable receptor-targeted ligands using different methods results in the formation of targeted nanoparticles with improved therapeutic response and minimized off-target side effects by prolonging their circulation time in blood, increasing target specificity, cellular uptake, and drug accumulation in the tumors (escaping lysosomal degradation and enhancing stimuli-responsive drug release) (**Figure 1**) [17, 19]. Depending on their application, nanoparticles are functionalized with different targeting ligands either by directly conjugating ligands to PEGylated nanoparticles through post-insertion technique or by covalent grafting on the surface of the nanoparticles. In this context, surface functionalization of nanoparticles with antibodies, peptides, folic acid, aptamers has been extensively studied. This prompts scientists to design and engineer nanoparticles for selective targeting and high retention in the tumor tissue rendering minimal toxicity to the vital organs [19, 20].

3. Mechanism of targeted drug delivery using nano-carrier

Nanoparticles play a vital role in promoting intracellular delivery of enclosed therapeutic agents and increase their retention in the different pathological tissues compared to other therapies [21]. Like normal tissues, tumors need nourishments by means of food and oxygen and a capacity to remove metabolic excretes and carbon dioxide. Diverse patterns of tumor-associated neovascularization, obtained by angiogenesis, cope with these demands. Primary conservative treatment modalities

involved in cancer treatment are surgery, radiotherapy, and chemotherapy, while additional therapies such as immune therapy, targeted therapy, and hormone therapy are chosen depending on the type of tumor [22]. On the other hand, the failure of chemotherapeutic drugs to specifically target cancer tissue hinders many treatment modalities. It is habitually faster and economically cheaper to design an existing drug to encapsulate in a delivery system a more effective way to superior targeting of tissues than to invent a completely new one. The drug delivery mechanism can be classified into passive and active, respectively.

3.1 Nanoparticle drug delivery by passive targeting

Passive targeted drug delivery mainly depends on the physicochemical attributes of the NMs, such as shape, diameter, surface potentials, and pathophysiological conditions of the disease microenvironment. Intravenously injected drug encapsulated NMs tend to disperse throughout the body evenly [23]. However, unlike normal tissues, tumor cells tend to take up particles of a definite diameter to a greater extent than healthy cells due to the arrangement of capillary endothelial cells, accumulating extravasated molecules in the interstitial spaces poor lymphatic drainage increases the permeation and accumulation of drug-mediated NMs. This type of NMs accumulation in the tumor region is known as the EPR effect [1, 2]. The EPR effect is influenced by physicochemical attributes of NM including particle diameter, shape, and surface potentials greatly influence the circulation time, penetration speed, tumor localization, and intracellular internalization.

Particle diameter plays a critical role in achieving effective drug delivery as it enhances permeation and circulation time and reduces renal clearance. For example, phagocyte cells facilitate larger particle uptake, while non-phagocytic cells favor the uptake of smaller particles. PEGylated NPs reduced plasma protein adsorption on their surface and reduced hepatic filtration when their size is smaller than 100 nm [24]. Particle diameter with 20–200 nm effectively enhances the permeation in both hyper-permeable and poorly permeable tumors, and particles with less than 6 nm avoid renal clearance. The NPs surface potentials could play a vital role in circulation and cellular localization [24, 25]. NPs with positive surface potentials such as cationic liposomes induce non-specific interactions with blood components and aggregation of liposomes results in a reduction of EPR effect and increased renal clearance. However, positively charged NPs are more readily taken up by cancer cells. Whereas anionic and neutral surface potential-bearing NPs circulate longer in the blood circulation [1, 2, 24].

Besides, Polyethylene glycol (PEG) polymer is used as a stabilizer (stealth liposomes) that increases the circulation time in blood up to 24–48 hours and improves *in vivo* stability [26]. PEG-coated liposomes induce the 'steric stabilization effect' by creating hydrophilicity on the surface of liposomes that shield surface charge and increases the repulsive forces between liposomes and blood components. Thus, it prevents aggregation of liposomes and opsonization by the reticuloendothelial system, macrophages, mononuclear phagocytic cells and prolongs their systemic circulation. On the other hand, PEG-coated liposomes induce PEG-specific IgM antibodies, enhancing hepatic uptake and rapid clearance of liposomes from systemic circulation on subsequent administration. PEG corona produces steric hindrance with tumor cells that prevent effective internalization, which could be minimized by using short PEG chains with molecular weight less than 1000 Da or by designing PEG with enzyme-cleavable bound or tumor-targeting ligands [20, 26].

To investigate the influence of shape on the cellular localization of NPs, Li et al. conducted large-scale molecular simulations to evaluate different NP geometries with identical surface area, ligand-receptor interaction strength, and PEG grafting density. They observed that spheres exhibited the fastest internalization rate, followed by cubes, while rods and disks were the slowest. Many liposomal formulations have received clinical approval, like Doxil, Abraxane, etc. However, nanoparticles grafted with PEG prolong the systemic circulation of the particles and induces the EPR effect in tumor cells, but lack of target specificity often results in reduced therapeutic efficacy [27]. Because of that, more than 95% of passively targeted formulations fail to go bench to bedside.

3.2 Nanoparticle-based drug delivery by active targeting

An ideal nanoparticle delivery system should be proficient at reaching, recognizing, and delivering its payload to determined morbid tissues and avoid drug-induced toxicity to healthy tissues [7]. Therefore, functionalizing specific targeting moieties on the surface of nanoparticles is the most usual plan. Nanoparticles are functionalized on their outer surface by targeting moieties such as small molecule ligands, monoclonal antibodies, aptamers, cell-penetrating peptides, and proteins that are internalized into morbid cells by interacting with cell surface receptors like folate receptors, transferrin receptors, tyrosine kinases like EGFR, and so on [28] (**Figure 1**). Cell surface receptors that are significantly overexpressed in diseased cells, compared to normal healthy cells, provide a potential target for the design and development of actively targeted drug delivery and help to reduce off-target effects [7, 17]. These ligand moieties can interact with target-specific diseased cells and protect nanoparticles from enzymatic demolition.

Targeted drug delivery significantly minimizes the toxicity and induces patient compliance with less frequent dosing. Active targeting depends on ligands bound to the NP surface to improve their uptake selectivity and protect NPS from enzymatic destruction. The main principle of active targeting involves functionalizing an NP with a ligand that binds to a molecule overexpressed on cells. Ligands with a high binding affinity to a specific cell type exhibit higher delivery efficiency. One important thing to consider is that healthy cells still express the same molecule, and as healthy cells greatly outnumber, the chances of NPs missing their target will also increase. An intelligent selection and functionalization with multiple ligands can effectively mitigate the problem. Apart from this, active targeting mainly determined the kind of nanoparticle carrier, ligand targeting specific receptors, functional agents used for linking a ligand to the nanoparticles, hydrophilic polymers, and encapsulated active ingredients [28, 29].

Targeting tumor cell surface receptors is a common approach in active targeting. Nanoparticles were linked with targeted ligands for targeting specific cell receptors and thus upregulated the intracellular localization and therapeutic efficiency. Liposomes are conjugated with antibodies, a Y-shaped glycoprotein, or its fragments often termed as immunoliposomes, increasing the specificity of liposomes by targeting antigen-presenting cancer cells, which undergo endocytosis and destroy cancer cells followed by immune system clearance [28]. Folate receptors are membrane proteins overexpressed by various tumor cells. Folic acid is a ligand for targeting folate receptors, which pose high affinity, stability, and conjugation capacity [30]. It is conjugated with nanoparticles and a PEG spacer that inhibits steric hindrance between the cells and liposomes, which helps to increase

cellular uptake and drug delivery of folate-targeted anticancer drugs. Targeting folate receptors with folic acid ligands helps deliver therapeutic and imaging agents effectively to the requisite site. Endothelial growth factor receptors (EGFR) over-expressed in solid tumors like non-small cell lung cancer, colorectal, squamous cell carcinoma of the ovary, kidney, head, neck, pancreas, prostate, and breast cancers can help in designing EGFR targeted drug delivery system. Antibody fragments used for targeting EGFR are functionalized on nanoparticle surfaces in order to acquire high targeting specificity [31]. Fibroblast growth factor receptors are over-expressed in cancers like lung, prostate, bladder, etc. Several groups have reported remarkable interaction of FGFs conjugated liposome with FGFR and discussed in detail [32, 33]. Overexpression of CD44 is observed in cancers like leukemia, ovarian, colon, gastric, pancreatic, and epithelial cancers. Hyaluronic acid acts as a ligand for CD44 and is used to deliver gemcitabine and DOX encapsulated within the liposomes [34].

Targeting the tumor microenvironment is another approach in active targeting, and one aspect is targeting the tumor vasculature instead of the tumor. This approach helps in the targeted destruction of neo-angiogenic blood vessels essential for tumor growth and metastasis [29, 35]. Vascular endothelial growth factor receptors (VEGFR) play a significant role in tumor angiogenesis and vascular permeability and regulate other aspects of tumorigenesis. Bevacizumab, a monoclonal antibody approved by USFDA, is used as an anti-human VEGF for targeting VEGFRs and FGFRs tyrosine receptors for active targeting [29]. Vascular cell adhesion molecules (VCAM-1) are cell adhesion molecules (CAMs) present on the endothelial cells responsible for inflammation. VCAM-1 is overexpressed in cancers like non-small cell lung cancer and tumor vasculature. Anti-VCAM and Fab-conjugated liposomes have high cellular uptake into Human Umbilical Vein and Endothelial Cells (HUVEC) compared to conventional liposomes [36].

Matrix metalloproteases (MMPs) are calcium-dependent endopeptidases involved in remodeling extracellular matrix, tumor invasiveness, and metastasis by modulating the formation of new blood vessels [37]. Conjugating MMP-2 cleavable peptides to liposomes loaded with cell-penetrating peptides increase the tumor selectivity. αβ-integrins are the heterodimeric transmembrane glycoproteins that facilitate the adhesion of endothelial cells with adjacent tissue and blood vessels. A tripeptide Arg-Gly-Asp (RGD) exhibited high specificity for αvβ3 integrin helps in developing integrin targeted liposomes, which inhibits adhesion and angiogenesis in the tumor microenvironment (TME) [38]. Active targeting amends the intuitive patterns of a nanocarrier, directing to the specificity of the pathological tissue. In contrast, passive targeting delivery depends on the natural distribution of the therapeutic motifs and the EPR effect. Both the targeting mechanisms depend on blood circulation and the location of initial drug delivery. However, rare commercial advances are made using actively targeted NPs [39].

4. Nanomaterial and their application from biological analysis

4.1 Nanomaterial-driven faster and more accurate cell analysis

Early detection and diagnosis can play a pivotal role in the battle against many diseases. Scientists harness the unique attributes of nanomaterials to generate novel molecular contrast agents for *in vivo* imaging, sensing, measuring response to therapy,

and liquid biopsy to study disease initiation, progression, and therapeutic response. Nanotechnology has a spacious range of accurate cell analyses. As described above, nanotechnology facilitates the development of desired formulations for individual cell analysis and their specific treatment applications, developing only one of its kind of applications for cell sensing/sensors, imaging, delivery, and diagnosis [39]. Since the importance of accurate cell analysis for nanoparticles is the latest approach, there is a big void for more discoveries and optimizations in various bio-applications.

4.2 Nanomaterial and *in vivo* imaging

The main lacunae in cancer treatment are a late diagnosis. The resolution of current imaging methods is low and can detect cancers at the late/ advanced stage or metastasized. A tissue biopsy can only help physicians to ascertain the tumor type and characteristics. Detection becomes even more challenging when metastatic modules and micrometastasis need to be identified. *In vivo* imaging enables us to non or minimally-invasively delve deep into the patient's tissue and is becoming increasingly popular for basic research and clinical applications. *In vivo,* molecular imaging focuses on obtaining spatiotemporal information about molecules of medical interest or biomarkers within a living body in real-time. Molecular *in vivo* imaging relies on contrast agents or medium that increases the contrast of physiological structure and enhances the sensitivity of detection. Different contrast agents are used for different *in vivo* imaging techniques including, radiocontrast, magnetic resonance imaging (MRI) contrast, ultrasound contrast, and optical contrast agents [40]. Precision diagnostics is dependent on high-resolution and high-contrast images. Nanomaterials are critical players in the generation of advanced contrast agents or media. Imageable nanoparticles can be classified based on their applications in nuclear, magnetic, optical, and acoustic imaging modalities. Moreover, NP-based contrast agents may be designed to integrate multiple detection modules and target specific cells. The advantages of nanoparticle-based contrast agents include enhanced specificity, increased photo and chemical stability, longer circulation time, engineered clearance pathways, and multimodal applications. The main *in vivo* imaging modalities include MRI, computed tomography (CT), positron emission tomography (PET), single-photon emission computed tomography (SPECT), ultrasonography (US), near-infrared fluorescence (NIRF), and two-photon intravital microscopy [41–43].

4.3 Nanoparticles as bio-sensors

By virtue of their unique properties, NPs make them ideal for their use for nano bio-sensing applications with enhanced sensitivity. Nanoparticles are widely used for detecting cells and pathogens, separating pathogens, recognize different biological substances, and detecting molecular and cellular functions [41, 42]. Accurate and professional separation of desired cells from the composite of various cell mixtures is essential for numerous biological applications. Nanoparticles have been investigated as a promising and very sensitive tool for the specific identification of cells. Identification and incarceration of metastatic cancer cells in the circulation can help understand and a strong analytical biomarker for various metastatic cancers, which can change the patient's prognosis. Nanoparticle-based methods are more frequently used for the identification and capture of metastatic circulating cancer cells. In this technique, magnetic nanoparticles were used to specifically track and separate the cells by using a

ligand-receptor-based mechanism [42]. These techniques can also be used for the white blood cells with an anti-CD45-APC as a nanoparticle targeting ligand [44].

Additionally, various nanoparticle-based technologies have been investigated as a sensor for the identification and selection of various pathogens. The most frequently used method for finding bacteria is magnetic biosensors that involve immunological mechanisms using magnetic nanoparticles functionalized with antibodies against surface antigens. Many researchers have been utilizing small molecule tethered nanoparticles to analyze the bacteria successfully. Magnetic glyco-nanoparticles mediated particles could detect bacteria within 5 minutes, including subtraction from the sample by the bacterial interaction with carbohydrates on mammalian cell surfaces [41].

4.4 Nanoparticles as imaging agents

Nanoparticles have been investigated as imaging agents due to their exceptional physicochemical attributes for various biomedical applications such as cancers and cardiovascular diseases. Fluorescent labels can be easily conjugated to the surfaces of the nanoparticles by various chemical methods to design a wide range of imaging agents for dynamic *in vitro* and *in vivo* cellular imaging [45, 46]. Due to their passive and active targeting nature, nanoparticles can easily identify their specific biomarkers and accumulate at high concentrations in the targeted tissue. The high capability for nanoparticle modification and retention properties in the specific tissue region empowers their utilization as imaging amplifiers. Quantum dots are the most promising fluorescent labels for cellular imaging among all nanoparticles due to their inherent near infra region light emitting nature, reducing autofluorescence [47].

RGD peptide conjugated self-emitting quantum dots can be used for specific integrins highly expressed in tumors. The targeted nanoparticle has been examined for complex imaging competence, like imaging various molecular targets using different spectral emissions specific nanoparticles. Recently, nanotechnology has been used for imaging metastatic tumor cells in circulation, tumor cells, and their vasculature, stem cells, and lymph nodes [48]. Che et al. designed shortwave infrared window (SWIR)-responsive QDs for bone-specific real-time *in vivo* and *ex vivo* imaging and could visualize the significant bone structures Balb/C nude and Balb/C mouse [49]. The use of specific nanoparticles can help accurately decipher and image the gram-negative and gram-positive bacteria. Due to their fluorescence characteristics and specific bacterial cell wall interactions, they can be used in a wash-free fashion in bacterial imaging, which is significant for health care, food processing, and medical hygiene.

4.5 Application of nanoparticles in theranostics

Theranostic NMs are designed by the consolidation of diagnostic and therapeutic abilities in one biodegradable nanoparticle [50]. Novel theranostic materials should have the following properties; i) highly compatible with biological entities, ii) proficiently and precisely accumulate in desired morbid tissue, iii) describe the biochemical and morphological attributes of maladies, iv) exhibit minimal toxicological effects, v) and deliver a sufficient amount of therapeutic agent. Several techniques have been used to functionalize the surface of nanoparticles for theranostics use. Surface functionalization may include imaging agents, drugs, therapeutic cargo, nucleic acid, and contrast agents by either chemical functionalization or by biofunctionalization. Chemical functionalization depends on chemical cross-linking, while biofunctionalization of nanoparticles relies on bioinspired ligands obtained from

natural phytochemicals). The use of nanotechnology offers a promising alternative for the diagnosis of various cancers. Various investigations convey that nanoparticles could be engineered for advanced diagnostic agents to detect cancers [51]. Double drug encapsulated liposomes can be functionalized to enhance theranostic efficacy [51, 52]. Multifunctional Metal nanoparticles can serve as a unique platform for cancer theranostics. The range of use of metal nanoparticles includes MRI imaging, biological catalysis, magnetic hyperthermia, magnetic drug delivery, photo-responsive drug delivery, and cell separation. Metal nanoparticles, including, Polymer-NP constructs containing Gd3+ complexes, Fe3 + — terpyridine complexes, and polymeric shell-based contrast agents, are widely studied for their theranostic use as MRI contrast. Magnetic particle imaging (MPI), a novel imaging technique, is based on the analysis of iron oxide NPs in response to a magnetic field.

Cheng et al. used GE11, a novel peptide with EGFR binding affinity and complexed with doxorubicin-loaded liposomes, and observed higher liposomal uptake and accumulation than, unconjugated liposomes using NIRF [53]. In another study Song et al. designed a multifunctional targeting liposome for targeting lung cancer. Octreotide (OCT), a synthetic 8-peptide analog of somatostatin, was used to surface coat the liposome for enhanced binding with the somatostatin receptors overexpressed in a subset of tumors. Double anti-cancer drug (Honokiol and epirubicin) co-encapsulated liposomes showed enhanced OCT- somatostatin receptor binding and *in vivo* response [54]. Cittadino et al. designed a theranostic long-circulating liposome with co-loaded prednisolone phosphate and an amphiphilic paramagnetic gadolinium contrast agent [Gd-DOTAMA(C18)(2)] for MRI monitoring of melanoma. The theranostically engineered liposomes showed long-term MRI-based detection without a loss in drug action [51]. The theranostic nanoparticle could assist in the patient's pre-selection, a prediction for responding to nanomedicine therapy. Moreover, nanomedicine-treated patients could be monitored throughout treatment duration while using nanomedicine formulations [39].

5. Biosafety and bioethics issues in handling mammalian cells

Biosafety is a notion that requires protecting human health and the surroundings of pathogenic and genetically modified mammalian cells or organisms used in the research. Mammalian cell culture is identified as a shelter for infectious etiologic substances, and it should change the compliance with containment measures recommended for the etiologic agent itself. The utility of cell cultures comes under the preview of a range of regulatory provisions that consider the estimation of biological risks. Genetically modified mammalian cell cultures were used in different continents; in that case, a bio-safety assessment should be regulated. The major guidelines issued to mitigate the biological risks for the users and environment are mainly by the World Health Organization; the Centers for Disease Control and Prevention, and the Swiss Expert Committee for Biosafety. Several countries or geographical zones have different directives; for example, in Europe, genetically modified research was brought into the regulatory provision (Directive 2009/41/EC). Mammalian cell culturing activities focusing on developing pharmaceutical drugs are covered by the Regulation (EC) No 726/2004 and its amendment laying down actions for the authorization and direction of medicinal goods for human and animal use. 3D cultures, especially organoid culture systems, are regularly used for disease modeling and studying nanomaterial-based physiological effects. Human Pluripotent stem cell-derived organoids are being

generated from various human cell types and need better bio-safety and bioethics assessment. It must be ascertained that rules focusing on extenuating the biological risks for laboratory researchers, public health, and the environment falls under the preview one or several regulatory provisions based on biological risk assessment. Here, we are going to address the bio-safety issues involving mammalian cell cultures.

5.1 Bio-safety assessments of mammalian cell cultures

Biosafety refers to the way of protecting scientists, the health of other humans, and the environment from the probable side effects of microorganism, pathogenic, and genetically modified organisms and cells from human and mouse backgrounds. Laboratory biosafety uses safety principles and techniques to minimize the health hazard from accidental exposure or unplanned spillage while using infectious agents, toxins and other biological hazards in the laboratory setting. The bio-safety assessments applied to mammalian cells depend on a systematic assessment of the intrinsic attributes of the mammalian cultures like genetically modified cells and contaminated or intentionally infected with pathogens. **Figure 2** shows a summary of the biosafety assessment and management process that is followed while handling cell culture-based experiments. This also considers an exposure analysis, which means that type of exploitation carried out with the cultures should be considered. The risk analysis of cell cultures that carry the pathogens follows the same methods for analyzing pathogens themselves. Primarily, the inclusive depiction of major pathogens is measured by the subsequent guidelines (i) pathogenicity and the infectious dose (ii) mode of transmission, (iii) host range, (iv) the epidemiology, potential reservoir and vectors, and the ability to zoonosis (v) the stability and the resilience of the pathogens in the surroundings.

Moreover, information related to the physicochemical properties of the pathogenic organism is considered, such as (i) susceptibility to disinfectants, (ii) physical

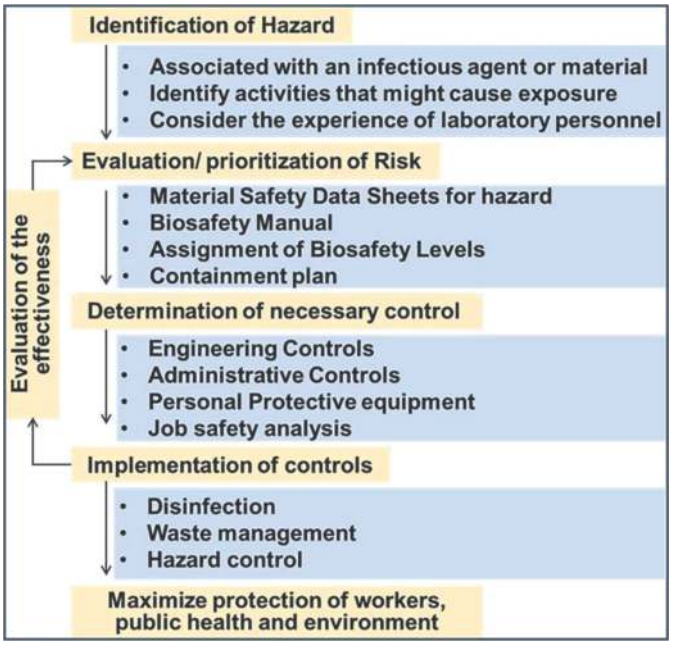

Figure 2.
Flow diagram illustrating the summarizing the biosafety assessment and management process while handling cell culture-based experiments. Flow chart is inspired by reference [55].

inactivation, and (iii) drug susceptibility (e.g., sensitivity and known resistance to antibiotics or antiviral compounds). Lastly, aspects related to the disease caused by the pathogen are also to be taken into consideration. This includes (i) the availability of effective prophylaxis, (ii) the availability of efficient therapy, and (iii) any reported case of laboratory-acquired infections (LAIs). Even though underemphasized, several LAIs of mammalian cell cultures (or having virus suspension) has appeared. Among all, the exposure to vaccinia viruses amplified in mammalian cell cultures causes infections to laboratory researchers. Guidelines have been developed recently to work cautiously with vaccinia viruses and take a count of LAIs relating to this virus [55].

Understanding and having a complete analysis of the intrinsic infections of cell cultures help to perform well and safe mammalian cell culture. To assess biological risks connected with the mammalian cell cultures, three intrinsic properties related to cell cultures should be considered: the species of origin, the cell type or type of tissue (the organ of origin of the cell line), and the status of the culture. Correspondingly, mammalian cells other than human cells render less risk; still, some infectious agents are proficient at crossing one species to another species, leading to zoonosis. Highly reported infections of viruses comprise hantavirus, hemorrhagic fever viruses, bird Influenza virus, and severe acute respiratory syndrome (SARS) associated virus. Primary cell cultures are created from organ tissues. Highly characterized mammalian cells give the lowest risks compared to primary cultures or less characterized cell lines. Mammalian cells originating from different laboratories without having any proof of identity may cause cross-contamination and pathogen spreading problems, and thereby proper risk assessment and cell characterization are warranted [55, 56]. Several techniques are available for the bio-safety assessment, like RT-PCR, flow cytometry, cytogenetic analysis, DNA fingerprinting, and iso-enzyme analysis. Adventitious contagions of mammalian cell cultures are a vital problem for any activity that involves cell culturing. Contamination agents for cell cultures are bacteria, fungi, mycoplasms, parasites, viruses, prions, and even other animal cells. Modulated experimental results suggest that they spoil the cell cultures. Bio-safety point of view modified mammalian cell cultures for laboratory research, production purposes, or diagnosis purposes they may give support for contaminating materials that cause harm to human health.

5.2 Bioethics and mammalian cell culture

The futuristic technologies in bio-medicine are changing the current concepts and opening up new dimensions. Interestingly as new optimistic channels are opening and expanding, the issues of bioethics are becoming accurate and pertinent. Bioethics is the use of ethical principles in the field of medicine and healthcare. The rational application of ethics in evaluating mammalian cell culture-based experiments is highly warranted, especially during the emerging waves of change in biomedicine. Increased International cross-connection to facilitate open discussion in bioethics and related fields across cross-cultural aspects in bioethics is vital [57]. Several relevant questions arise regarding the private and sensitive use of source data for cells, moral concerns regarding the uses of embryonic and fetal tissue, genetic manipulation, gene therapy, mixing of animal and human cells, tissue banking, legal and intellectual properties associated with *ex vivo* tissue-engineered cell-based products, an extension of human-ness, etc.

Regarding the humane use of animals, the National Institutes of Health has issued policies as mentioned in the Public Health Service Policy on Humane Care and Use of Laboratory Animals. FDA Human Tissue Task Force and the Center for

Biologics Evaluation and Research (CBER) regulates the use of human cells or tissue for implantation, transplantation, infusion, or transfer into a human recipient. The International Society for Stem Cell Research (ISSCR) has also released guidelines for stem cell research and clinical translation. The United States Congress and state legislatures are instrumental in creating laws concerning bioethics. Several professional bioethics organizations, including the American Society for Bioethics and Humanities, American Society for Law, Medicine, and Ethics, Canadian Bioethics Society, provide a platform for discussion over bioethics [57]. Several public institutions supported by academicians and researcher-based initiative for propagating public dialog plays a vital role in educating the masses.

6. Significance of IPR on industrial and academic scale

Intellectual property rights (IPR) prevail in any primitive design of the human brain, such as methodical design. IPR mentions the lawful rights agreed to the designer for guarding his innovation for a definite period. These lawful rights grant special rights to the originator or his lender to exploit his idea for a specific period. It is well established that IPR participates in the financial system. It is furthermore overwhelmingly recognized that the intellectualism linked with the originality must be agreed due to value so that products come out of intelligence. The importance of the producer of the technology has turn into lofty and consequently guard the information against unauthorized persons, the use has become a measure, at least sometimes, that would make sure revitalization of the research, investments in developing the technology. IPR helps to look after funds, time, capital, endeavor invested by the producer of an intellectual idea; as a result, IPR, in this way, encourages the profitable encouragement of a realm by encouraging positive competition and heartening trade and industry [58].

The industries have reputations in discussions about IPR strategies, and they are in the face line for controversies about the association among IPRs, R&D incentives, cost, and right to use to supplies [59]. Although, some discussions on the critical issue are relatively little practical proof to support developing IPR policy. This experimental evidence on IP and products inspect practical issues are the primary sources of the data. The industrial sector is composite and much synchronized in the majority of economies. Looking cross-nationally, the contrast among the countries in their perspective on these essential policy affairs generates some additional provocations. In a cosmopolitan industry having control over research and development conveniences in many countries, anticipating a successful transnational technology, goods are raised and developed internationally and are commercialized worldwide. Still, retails are nationalized, with no considerable uniformity across the nations in IPR authorities and various public health care organizations. IPRs may shore up significant discrepancies to price across the nations in returns and demand to prices. These discrepancies in the prices may potentially develop new local and global disagreements. Prominently, for any nation, the essential exchange in IPR regulation options is incredibly dependent on the organizations and function of its health care system.

While having a commendable collaboration, the complete fulfillment of a patent portfolio is to give equal rights for industry and academic institutions. In many countries, research organizations pursuing research in academic institutions, despite their most important work in society as a generator of the intellectual idea, the main concern is to be to deal with IP in a proficient mode. All academic

institutions must become accustomed to this development to successfully fulfill the responsibility entrusted to a national or regional innovation ecosystem.

On the supply side, goods safety, supervision of manufacturing, and legal frameworks leading technology transfer among public-funded academic institutions and money-making industries playing an equal role in determining competition. Providing IPR policy to academic institutions has a favorable outcome and various settlements for shareholders. The most significant overarching advantage of these IPR policies was pronounced increases involvement in improving the global innovation performance, i.e., ultimately leading to improving the marketable products and processes. The development of spin-out companies from universities is also growing at a faster rate. The critical part is that the university should own the background IP. Then a resource of external financial support is necessary to finance the start-up company. IPR affairs at academic institutions glow enormous meandering return impending for the national economy. Publishing articles regarding innovations play an essential role in the profession of academic scientists. Participating in knowledge transfer from academia to manufacturing industries can promote academic entrepreneurship. Moreover, these patents have precious information than other publishing articles. Thus, utilizing and increasing patent writing might be beneficial in scientific research. Appropriate IPR policies and tractable technology transfer professionals play a pivotal role in streamlining the necessary work-frame. Published patents improve the economy and reputation of the academic institutions as well as the researchers.

7. Conclusion

Nanomaterials, due to their nano-size and unique physicochemical properties, have contributed significantly to the advance of biomedicine. The scope of nanomedicine also relies on the intelligent engineering of different nanoparticles with tunable attributes to modulate their nano-bio communications for biomedical applications. Elucidation of nanoparticle interactions with biological systems will help find favorable physicochemical properties to enhance biocompatibility and therapeutic efficacy with no adverse effects. A complete toxicological evaluation of engineered nanomaterials is still inadequately understood, restraining the successful translation of nanomedicine. Nanoparticle surface functionalization with specific targeting moieties can effectively develop ideal nanoparticle delivery systems for various biomedical applications and targeted therapeutics. Hence, *in vitro* 2D and 3D cell culture systems can accelerate biocompatibility and biotoxicity studies to drive the disease-specific application of nanoparticles [60]. Nanoparticles are progressively used in a wide variety of cell and tissue-specific biological analyses, including cell analysis, in vivo imaging, biosensors, and theranostics. Hence the issue of biosafety and bioethics has become a vital issue while using mammalian cell cultures. This chapter summarizes the critical aspects of biosafety and bioethics associated with nanomaterial-associated studies.

In conclusion, MCC is an essential tool in modern-day biomedicine, and its applications are countless in the diagnosis and therapy of human diseases. Cell culture procedures are reliable, reproducible, and unbiased, but culturing the cells is complex at times. The vast opportunities to employ MCC procedures to address rudimentary and translational research queries have elucidated the essential attentions for setting up a cell culture laboratory. Especially 3D organoid culture methods have created a cellular environment that mimics the *in vivo* environment.

Genome sequencing, mapping, and annotating its genetic code have become a priority in biotechnology, especially intending to understand the interaction of nanoparticles and mammalian cells. Reporting and cataloging the identified gene sequences can be critical for the progress of science and also for disease-specific therapeutics. Nanotechnology-based research has contributed significantly to many scientific fields and associated industries. Hence nanotechnology, combined with the mammalian cell culture system, can result in a research solution and can deliver considerable benefits to society at large. Hence the importance of intellectual property rights for protecting the innovator's right over the discovery. A good understanding of the IPR policies and technology transfer protocol is vital. Academic institutions and government organizations can assist in creating a congenial platform for efficient policy management. A deeper understanding of nanoparticle-cell interaction and the design of futuristic nanocarriers can open up an era of next-generation therapeutics and theranostics.

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Abbreviations

CBER	Centre for Biologics Evaluation and Research
CT	Computed Tomography
EGFR	Epidermal Growth Factor Receptor
EPR	Enhanced Permeability and Retention
FGFR	Fibroblast Growth Factor Receptor
HUVEC	Human Umbilical Vein Endothelial Cells
IgM	Immunoglobulin M
IPR	Intellectual Property Rights

ISSCR	International Society for Stem Cell Research
LAIs	Laboratory-Acquired Infections
MMPs	Matrix Metalloproteases
MPI	Magnetic Particle imaging
MRI	Magnetic Resonance Imaging
NIH	National Institutes of Health
NMs	Nanomaterials
NPs	Nanoparticles
NRIF	Near-infrared Fluorescence
NT	Nanotechnology
OCT	Octreotide
PEG	Polyethylene glycol
PET	Positron Emission Tomography
QD	Quantum Dots
SARS	Severe Acute Respiratory Syndrome
SPECT	Single-photon Emission Computed Tomography
SRA	Sequence Read Archive
SWIR	Shortwave Infrared
TME	Tumor Microenvironment
US	Ultrasonography
USFDA	United States Food and Drug Administration
VCAM-1	Vascular Cell Adhesion Molecules-1
VEGFR	Vascular Endothelial Growth Factor Receptors

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